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AMENDMENTS TO THE DRAWINGS

The attached sheets of drawings are formal drawings for submission and replacement of the original sheets including Figs. 1-10.

REMARKS

Applicants appreciate the careful consideration and favorable treatment of the claims by the Examiner in which claims 16, 17, 20, 21, 32, 33 and 35-37 have been indicated as containing allowable subject matter.

Applicants submit herewith a set of clean formal drawings and therefore, the Examiner's objection to the drawings should be withdrawn.

Applicants also submit a cleaner copy of reference #26, Yoshioka et al.; however, Applicants are surprised that the submitted copy is illegible since Applicants' copy is clearly legible. It is requested that the Examiner initial reference #26 on the previously submitted PTO form 1449 and return a copy of the same to Applicants.

The objection to claim 24 is believed to be moot since all of claims 22-24 depend from new independent claim 20. Withdrawal is in order.

Claims 14, 15, 27, 28 and 34 stand rejected under 35 U.S.C. 112, second paragraph. With respect to claims 14 and 15, the dependency of these claims has been changed to claim 13 as the Examiner suggests. Withdrawal of this rejection is in order. Claim 27 has been amended to clarify the data associated with a chemical reaction occurring within the reaction vessel is collected and logged as a single data point for display on a corresponding graph. The specification makes it clear that when a graph is formulated, each reaction vessel is represented as a single data point on the graph. In view of the present amendment and the description in the specification, withdrawal of this rejection is in order. With respect to claim 28, Applicants have added the conjunction between parts (b) and (c) as the Examiner suggests. On this basis, withdrawal of the present rejection is in order.

Claims 1-8, 11-15, 18, 19, 22, 23, 26, 28 and 31 stand rejected under 35 U.S.C. 102(b) as being anticipated by Danssaert et al.

The Danssaert et al. reference is directed to a thermal cycler including a temperature gradient block that is configured for use a thermal block for PCR reactions. The specification does mention that in use, the thermal gradient block can be placed in a system that includes a robot arm for moving a reaction mixture between blocks. However, the reference does not disclose that the device is configured for conducting chemical reaction kinetics studies which requires an interface that is configured to do the same as disclosed in the present application.

Claim 1 has been amended to recite an automated apparatus for performing reaction kinetics studies. In the amended claim, the controller is configured such that both isothermal and nonisothermal temperature profiles can be performed in the same apparatus. Applicants respectfully submit that the cited reference fails to discuss or disclose an apparatus that is configured to operate in the aforementioned manner. On at least this basis, Applicants respectfully contend that the rejection of claim 1 should be withdrawn since one or more features recited in claim 1 is not disclosed in the cited reference. Reconsideration and allowance of claim 1 are respectfully requested.

Claims 2-15 and 27 should be allowed as depending from what should be an allowed independent claim 1.

Claim 16 has been amended into independent form by including the subject matter of claim 1. The Examiner has indicated that claim 16 contains allowable subject matter and therefore, Applicants respectfully submit that the present amendment to claim 16 places this claim in condition for allowance, which is earnestly solicited at this time. This is not a narrowing amendment since claim 16 originally depended from claim 1.

Claim 17 has been amended into independent form by including the subject matter of claim 1. The Examiner has indicated that claim 17 contains allowable subject matter and therefore, Applicants respectfully submit that the present amendment to claim 17 places this claim in condition for allowance, which is earnestly solicited at this time. This is not a narrowing amendment since claim 17 originally depended from claim 1.

Claim 18 has been amended to recite an automated apparatus having a controller with a user interface for inputting at least (1) a number of reaction vessels for the study; (2) a first predetermined temperature profile; (3) a predetermined study time period beginning with a start time and ending with a stop time, and (4) a selected kinetics model. The apparatus is configured so that the kinetics data is fitted to the selected kinetics model inputted by the user and is used to generate a representative temperature vs. time graph.

Applicants respectfully submit that the cited reference fails to disclose the above feature since the reference does not have an interface where the user inputs a selected kinetics model to which kinetics data associated with each reaction vessel is fitted to generate a representative temperature vs. time graph. Since this feature is not disclosed and is not even suggested by the cited reference, Applicants respectfully submit that a rejection under 35 U.S.C. 102(b) is improper and therefore, must be withdrawn. Reconsideration and allowance of amended claim 18 are respectfully requested.

Claim 20 has been amended into independent form by including the subject matter of claim 18. The Examiner has indicated that claim 20 contains allowable subject matter and therefore, Applicants respectfully submit that the present amendment to claim 20 places this claim in condition for allowance, which is earnestly solicited at this time. This is not a narrowing amendment since claim 20 originally depended from claim 18.

Claims 19 and 21-26 should be allowed as being dependent on what should be an allowed independent claim 20, as amended.

Claim 28 has been amended to recite that the second input which corresponds to a predetermined temperature profile is one of a nonisothermal temperature profile and an isothermal temperature profile. The method of claim 28 has also been amended to recite that the kinetics data is fitted to an inputted kinetics model.

Again, Applicants respectfully submit that the cited reference is silent as the inputting a kinetics model and to fitting kinetics data to said model and also to the use of temperature profiles

that are either of a nonisothermal or isothermal nature. Since, Applicants contend that these features are not disclosed or even suggested in the cited reference, amended claim 28 should be passed to issue.

Claim 32 has been amended into independent form by including the subject matter of claim 28. The Examiner has indicated that claim 32 contains allowable subject matter and therefore, Applicants respectfully submit that the present amendment to claim 28 places this claim in condition for allowance, which is earnestly solicited at this time. This is not a narrowing amendment since claim 32 originally depended from claim 28.

Claims 29-31 and 33-25 should be allowed as depending from what should be an allowed independent claim 32.

Claim 36 has been amended into independent form by including the subject matter of claim 28. The Examiner has indicated that claim 36 contains allowable subject matter and therefore, Applicants respectfully submit that the present amendment to claim 36 places this claim in condition for allowance, which is earnestly solicited at this time. This is not a narrowing amendment since claim 36 originally depended from claim 28.

Claim 37 should be allowed as depending from what should be an allowed independent claim 36, as amended.

The rejection of claims 9, 10, 24, 25, 29 and 30 under 35 U.S.C. 103(a) as being unpatentable over Danssaert et al. in view of Burrow et al. (U.S. Patent No. US 2002/0090320) is now moot in view of the present amendment.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

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Report

Isothermal and Nonisothermal Kinetics in the Stability Prediction of Vitamin A Preparations

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A nonisothermal method was applied to shelf-life estimation of commercial vitamin A preparations. The usefulness and limitations of the nonisothermal method, as predicted by computer simulation, were validated. Degradation of vitamin A palmitate followed zero-order kinetics, and non-Arrhenius behavior was suggested for the rate constant. The nonisothermal method predicted a higher shelf-life value than the isothermal method for a syrup preparation, and vice versa, for an injection preparation. Analysis of the results suggested that the nonisothermal method provides better estimates of shelf lives than the isothermal method when drug degradation does not follow the Arrhenius equation near ambient conditions.

KEY WORDS: stability prediction; nonisothermal method; isothermal method; vitamin A; shelf life.

INTRODUCTION

The use of nonisothermal kinetic methods for predicting drug stability is currently increasing because of the increasing availability of automatic temperature-controlling equipment and computers for estimating parameters (1–7). The reliability of the nonisothermal methods, however, remains to be clarified.

Recently we reported a statistical evalulation of nonisothermal shelf-life estimations of pharmaceutical products (8,9). An equation was developed which allowed direct nonlinear estimation of shelf lives (the time period required for a drug to degrade to 90%). The accuracy and precision of the shelf-life estimates obtained with this equation were evaluated by the Monte Carlo method with regard to the dependence on the experimental conditions such as temperature programs, sampling frequency, and sample numbers. The errors in measurement of drug contents and in temperature control were main factors limiting accurate shelf-life prediction. Further, the accuracy and precision of the estimates were determined mainly by the extent of drug degradation, the temperature changing range, and the number of experimental data at temperatures close to room temperature. Thus, typical experimental designs were proposed that provide reliable estimates of shelf lives and are applicable to various drug products with a broad range of shelf lives. The nonisothermal method according to these proposed temperature programs was compared to the isothermal method. The nonisothermal method was found to give smaller bias and variance of the shelf-life estimates than the isothermal method carried out at actual temperatures for a relatively long period. The nonisothermal method, however, yielded larger bias and variance of the shelf-life estimates than the isothermal method carried out at elevated temperatures for a short period. These comparisons were made assuming that drug degradation follows the Arrhenius equation. On the other hand, the nonisothermal method was superior when drug degradation does not follow the Arrhenius equation. The bias of the estimates brought about by neglecting the nonlinearity of the Arrhenius plots was much smaller in the nonisothermal method than in the isothermal method.

The purpose of this paper was to compare the proposed nonisothermal method with the isothermal method in shelf-life estimation of commercial preparations and to validate the usefulness and limitations of the nonisothermal method, which has been indicated by computer simulation in the previous studies. Syrup and injection preparations of vitamin A were chosen because they usually have relatively short shelf lives that can be estimated by a relatively short-period testing, and it is not necessary to consider the effect of humidity as required for the degradation rate of solid dosage forms.

MATERIALS AND METHODS

Kinetic Studies

Commercial syrup and injection preparations of vitamin A palmitate (purchased) were subjected to isothermal and nonisothermal stability testing. Syrups were transferred from the original bottles to test tubes with stoppers and, thus, rendered different in the ratio of the void (air) volume to the sample from the original product. Isothermal studies were carried out at four levels of temperature (40, 50, 60, and 70°C for the syrup preparation and 50, 60, 70, and 80°C for the injection preparation). In the nonisothermal studies, temperature T (°C) was a function of time t (weeks) as follows: $T = 25 + 0.004t^2$ or $T = 40 + 7t$. Samples were stored in a

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thermostat chamber (HIFLEX FX 2100, ETAC). At appropriate intervals, samples were removed and assayed. Degradation of the syrup preparation was also followed at 25°C to measure the shelf lives at 25°C and to validate the estimates obtained by the isothermal and nonis thermal methods.

Vitamin A Palmitate Assay by HPLC

Three samples were taken out at each sampling time and combined into one sample solution. After dilution with isopropanol, the sample solution was injected through a 10-μl loop to a column (ODS-80TM, 15 cm × 4.6 mm, TOSOH) maintained at 35°C. The mobile phase was a mixture of acetonitrile, methanol, and methylene chloride (7:3:3), which was delivered at a rate of 1 ml/min. The column eluate was monitored at 328 nm. The measurement was repeated three times.

Estimation of Shelf Lives

Shelf lives at 25 and 40°C [$t_{90(25)}$ and $t_{90(40)}$] were estimated by using the iterative technique of the damping Gauss–Newton method as described previously (8).

In the nonisothermal method, Eqs. (1), (2), and (3) for zero-order kinetics were used for calculation. Equation (2) was integrated numerically according to the Simpson's ⅓ rules.

$$C = C_0 \left[1 - \frac{0.1}{t_{90(25)}} \exp\left(\frac{E_a}{R \cdot 298}\right) \cdot I\right] \quad (1)$$

$$I = \int_0^t \exp\left(\frac{-E_a}{R \cdot T(t)}\right) dt \quad (2)$$

$$E_a = \frac{R \cdot \ln[t_{90(25)}/t_{90(40)}]}{1/298 - 1/313} \quad (3)$$

where C_0 and C are drug contents at time 0 and time t, $T(t)$ is the absolute temperature at time t, and E_a and R are the activation energy and the gas constant.

In the isothermal method, the estimation of shelf lives was carried out by using Eq. (4) for zero-order kinetics:

$$C = C_0 \left\{1 - \frac{0.1}{t_{90(25)}} \exp\left[\frac{E_a}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right] \cdot t\right\} \quad (4)$$

where T is the absolute temperature. A set of $t_{90(25)}$ and $t_{90(40)}$ estimates was calculated at one time from all the data observed at four levels of temperature (40, 50, 60, and 70°C for the syrup preparation). Another set was calculated from the data obtained at three lower levels of temperature (40, 50, and 70°C for the syrup preparation), as well as at two lower levels of temperature (40 and 50°C for the syrup preparation).

The estimation of the $t_{90(25)}$ or $t_{90(40)}$ from the data obtained at 25 or 40°C was carried out by using Eq. (5).

$$C = C_0 (1 - 0.1t/t_{90}) \quad (5)$$

Programs used for these estimations were written in BASIC and compiled (PC-9801 VMII, NEC).

RESULTS AND DISCUSSION

Figures 1 and 2 show the degradation time courses of

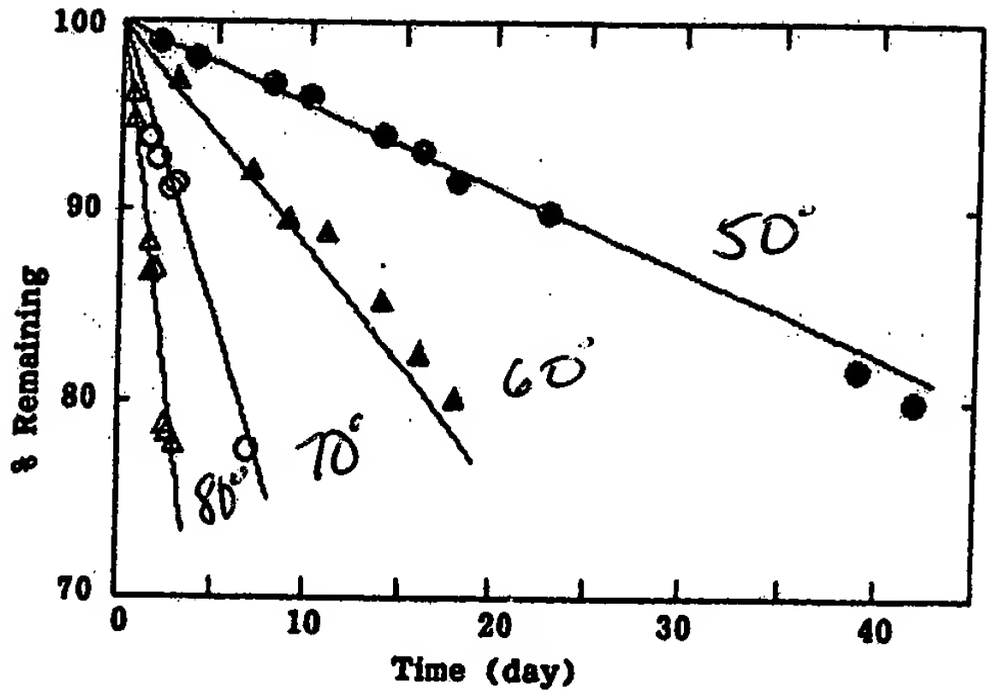

Fig. 2. Isothermal degradation of vitamin A palmitate in injecti n preparation. ●, 50°C; ▲, 60°C; ○, 70°C; △, 80°C.

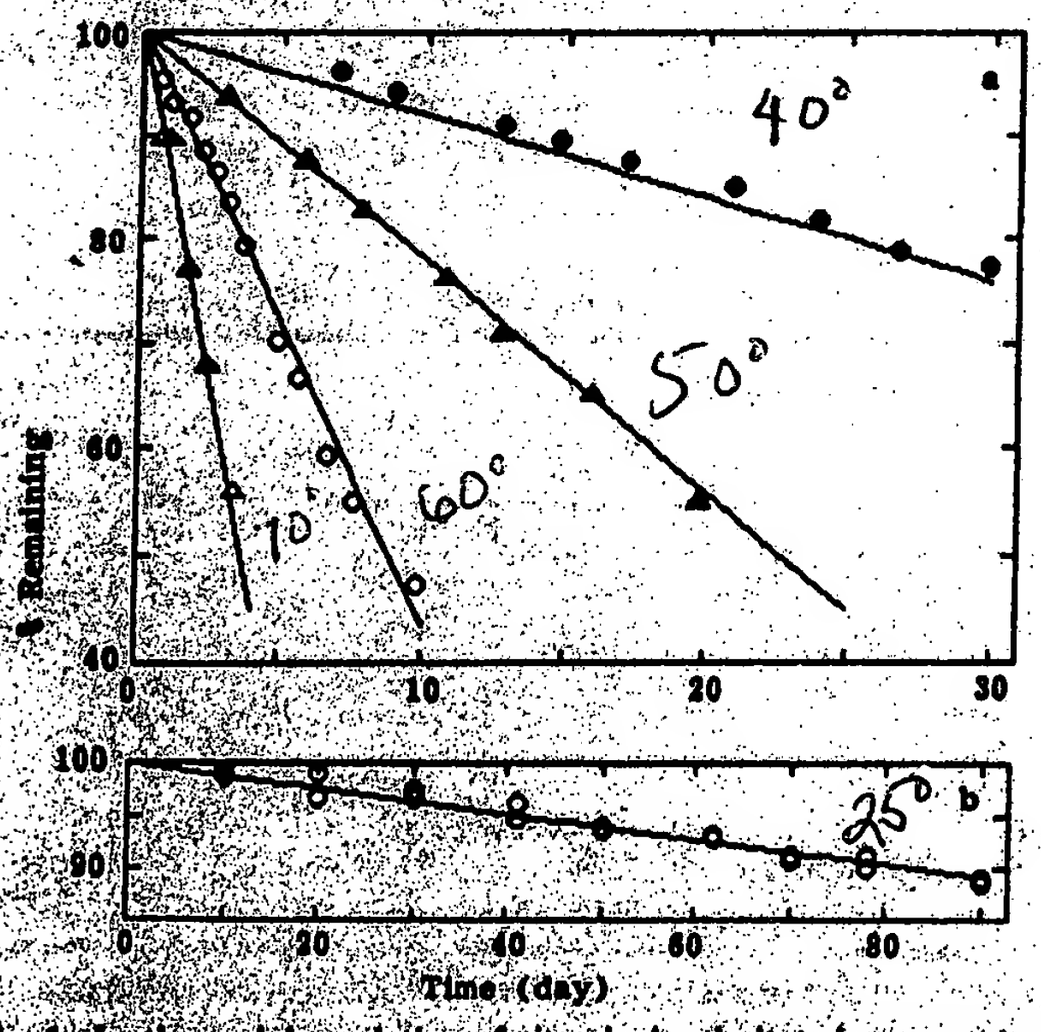

Fig. 1. Isothermal degradation of vitamin A palmitate in syrup preparation. (a) ●, 40°C; ▲, 50°C; ○, 60°C; △, 70°C. (b) ○, 25°C. See the text for the lines.

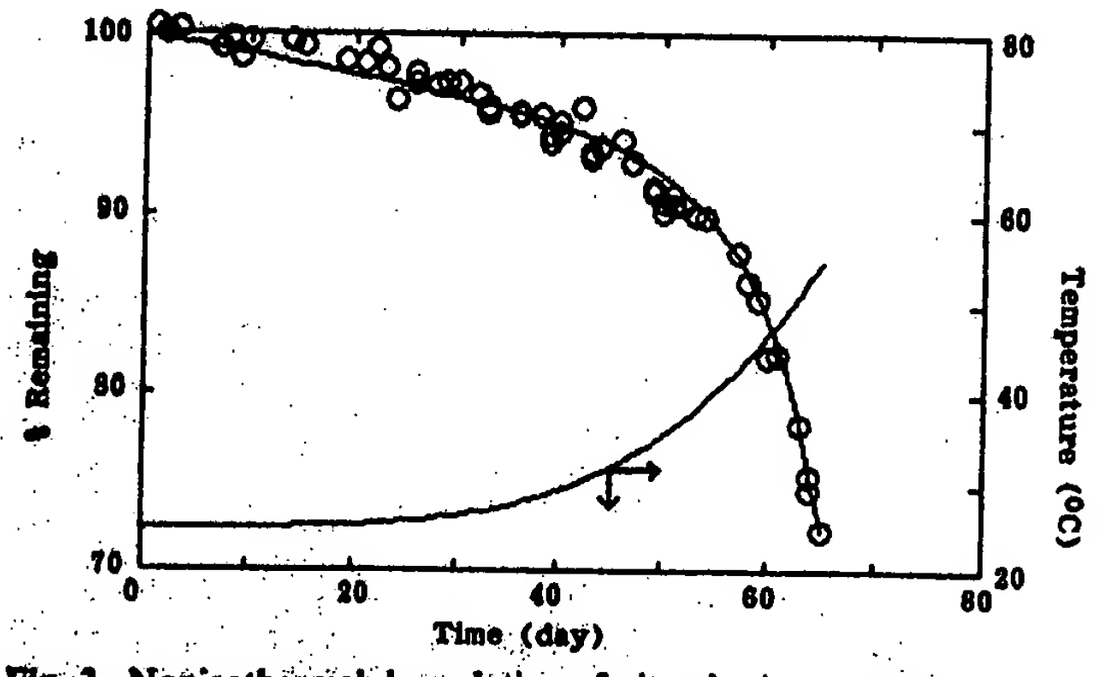

Fig. 3. Nonisothermal degradation of vitamin A syrup preparation. Temperature program: T(°C) = 25 + 0.004t(week)^4. See the text for the lines.

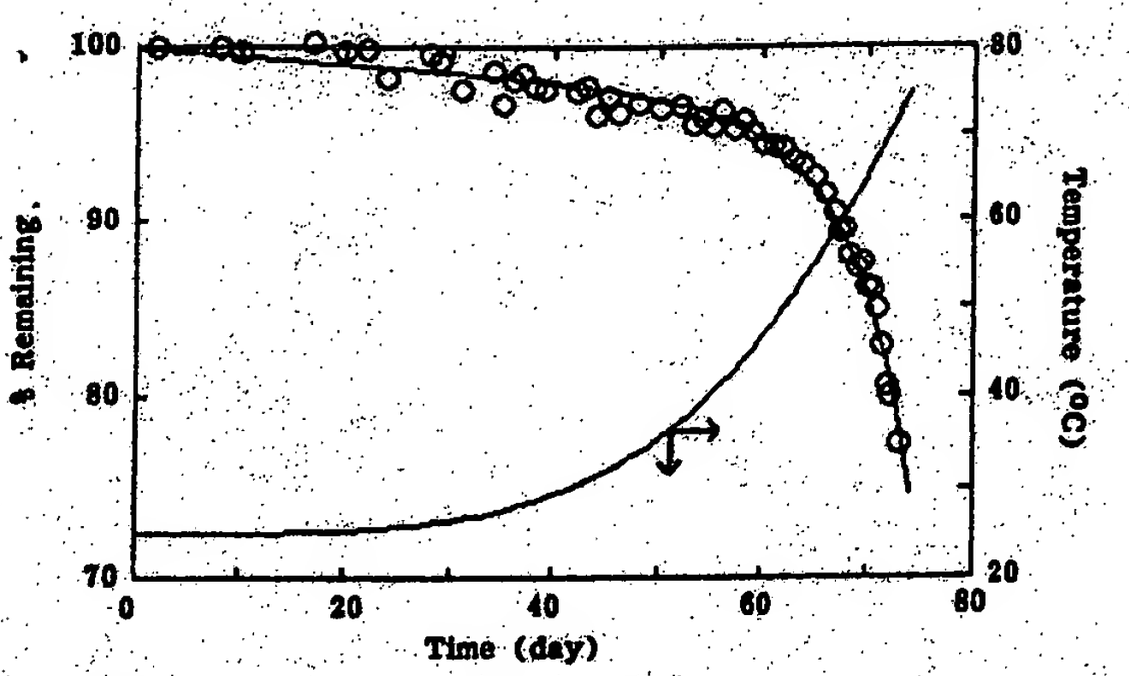

Fig. 4. Nonisothermal degradation of vitamin A injection preparation. Temperature program: $T(°C) = 25 + 0.004t(\text{week})^4$.

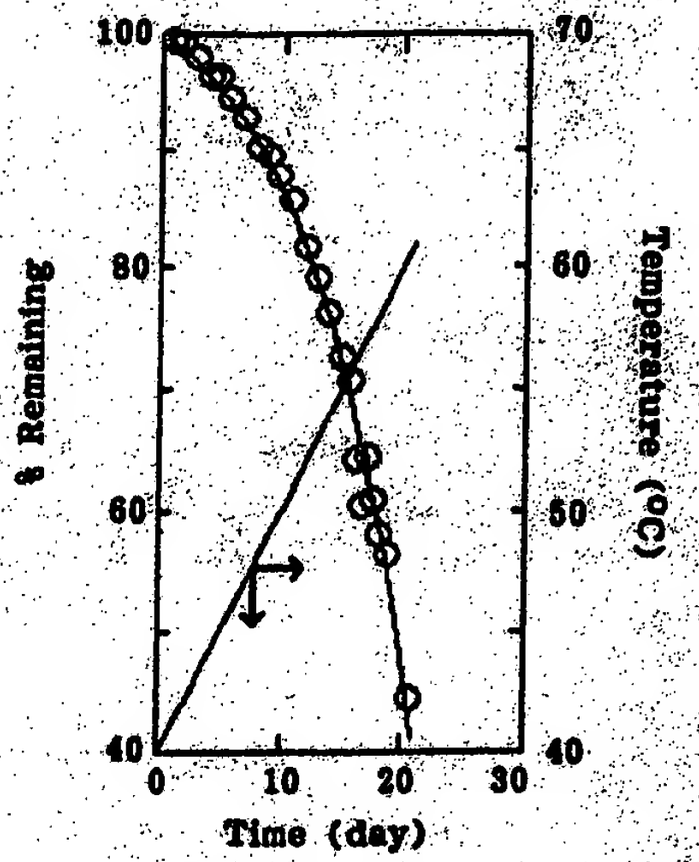

Fig. 5. Nonisothermal degradation of vitamin A syrup preparation. Temperature program: $T(°C) = 40 + 7t(\text{week})$.

vitamin A palmitate in syrup and injection preparations, respectively, observed under isothermal conditions. Figure 1 includes the degradation data observed at 25°C for the syrup preparation. Initial degradation appeared to follow zero-order kinetics rather than first-order or second-order in both preparations. Zero-order kinetics were assumed in the following analysis. The solid lines represent the regression curves calculated at one time using Eq. (4) from all the data observed at four levels of temperature, assuming that E_a is constant in the temperature range. The 40°C curve for the syrup and the 50 and 60°C lines for the injection removed from the experimental data. This suggests that E_a cannot be considered constant.

Figures 3 and 4 represent the degradation time courses of the syrup and the injection preparations, respectively, observed under nonisothermal conditions. The temperature program used ($T = 25 + 0.004t^4$) was one that has been proposed for products with relatively short shelf lives in the previous study (9) and is shown in Figs. 3 and 4. This program was selected since the shelf lives were indicated to be 1 year on the labels for both preparations. The solid lines represent the regression curves calculated by Eqs. (1), (2), and (3). For the syrup preparation, a shorter observation period of nonisothermal study was also carried out with another temperature program ($T = 40 + 7t$), and the result is shown in Fig. 5.

The shelf lives of the syrup and the injection preparations obtained by the nonisothermal method are shown in Tables I and II, respectively, in comparison with those obtained by the isothermal method. For the syrup preparation, $t_{90(25)}$ and $t_{90(40)}$ were also estimated from degradation data at 25 and 40°C, respectively. The estimates shown in Tables I and II were obtained by using the iterative technique based on the damping Gauss–Newton method. The standard deviation of each estimate is also shown in the tables.

In the case of the syrup preparation, the nonisothermal method using the proposed temperature program ($T = 25 + 0.004t^4$) provided the $t_{90(25)}$ and $t_{90(40)}$ estimates close to the values estimated from the experimental data at 25 and 40°C, respectively. On the other hand, the isothermal method carried out at 40, 50, 60, and 70°C gave much smaller estimates than those measured experimentally at 25 and 40°C. The estimates calculated from the isothermal data depended on which temperature data are used for the estimation. The

Table I. Shelf Lives of a Syrup Preparation Estimated by the Isothermal and Nonisothermal Methods

Method	Temp. (°C)	Shelf life (days) $t_{90(25)}$	Shelf life (days) $t_{90(40)}$	E_a[a] (kcal/mol)
Isothermal	40, 50, 60, 70	60.9 ± 1.9[b]	12.1 ± 0.2	20.1 (19.4–20.6)
	40, 50, 60	64.6 ± 2.5	12.4 ± 0.2	20.5 (19.7–21.1)
	40, 50	78.5 ± 3.7	13.2 ± 0.2	22.2 (21.2–22.8)
Nonisothermal	$T(°C) = 40 + 7t(\text{week})$ (40–60.7°C)	72.8 ± 9.4	13.0 ± 0.7	21.4 (18.9–23.5)
	$T = 25 + 0.004t^4$ (25–54.7°C)	83.0 ± 1.9	13.9 ± 0.2	22.2 (21.6–22.5)
Observed[c]	25	82.7 ± 1.8		
	40		13.2 ± 0.2	

[a] E_a calculated from the $t_{90(25)}$ and $t_{90(40)}$ estimates. The ranges in parentheses calculated by taking account of the standard deviation of $t_{90(25)}$ and $t_{90(40)}$ estimates.
[b] Standard deviation.
[c] Shelf lives estimated from experimental data obtained at 25 and 40°C.

Table II. Shelf Lives of an Injection Preparation Estimated by the Isothermal and Nonisothermal M thods

Method	Temp. (°C)	Shelf life (day) $t_{90(25)}$	Shelf life (day) $t_{90(40)}$	E_a[a] (kcal/mol)
Isothermal	50, 60, 70, 80	375 ± 22[b]	66.0 ± 2.4	21.5 (20.3–22.6)
	50, 60, 70	321 ± 28	61.1 ± 2.8	20.5 (18.8–22.1)
	50, 60	218 ± 13	51.5 ± 1.5	17.8 (16.7–18.9)
Nonisothermal	$T = 25 + 0.004t^4$ (25–72.3°C)	211 ± 8	47.8 ± 0.8	18.3 (17.7–19.0)

[a] E_a calculated from the $t_{90(25)}$ and $t_{90(40)}$ estimates. The ranges in parentheses calculated by taking account of the standard deviation of $t_{90(25)}$ and $t_{90(40)}$ estimates.
[b] Standard deviation.

estimate became smaller when the data obtained at higher temperature were included in the estimation. The estimates closer to the observed $t_{90(25)}$ could be obtained from the limited isothermal data at lower temperatures, although the standard deviation of these estimates became larger because of the decrease in the data number. These results suggest that E_a changes with temperature in the syrup degradation. The decrease in the $t_{90(25)}$ estimate (i.e., increase in k_{25} estimate, apparent rate constant at 25°C estimate) with higher temperature data indicates the E_a is smaller near 60 and 70°C than near 40 and 50°C. This E_a change, which is neglected in the estimation of $t_{90(25)}$ and $t_{90(40)}$, brings about large bias of the estimates (difference between the estimates and the real values). The effect of the E_a change on the estimate seems to be smaller in the proposed nonisothermal method than in the isothermal method, because the former method includes the data obtained at temperatures close to room temperature. The isothermal method without the data near room temperature resulted in underestimation of the shelf life. The nonisothermal method using a linear temperature program for a shorter period ($T = 40 + 7t$) also provided an underestimated shelf life from lack of room-temperature data.

In the case of the injection preparation, the nonisothermal method provided a lower value of shelf life than the isothermal method as shown in Table II. The estimate obtained by the isothermal method became larger, when the data obtained at higher temperatures were included in the estimation of shelf life. This is the opposite for the syrup preparation and suggests that E_a changes with temperature in the opposite way. It is suggested that E_a is larger near 70 and 80°C than near 50 and 60°C. The isothermal method, in this case, resulted in overestimation of the shelf lives. These results for the syrup and the injection preparations indicate the usefulness of the nonisothermal method in the estimation of shelf lives, when drug degradation does not follow the Arrhenius equation.

Differences in the kinetic behavior of vitamin A were observed between the syrup and the injection preparations. The degradation rates differ from each other and the E_a values change with temperature in opposite ways for the two preparations. These observations may be ascribed to the difference in the physical state of vitamin A in the two preparations—i.e., o/w emulsion for the syrup and w/o emulsion for the injection. The E_a change shown in both cases may be explained in terms of the change in solubility and micelle formation with temperature.

In conclusion, one of the useful characteristics of the nonisothermal method, which had been suggested by previous computer simulations (9), was validated in the stability prediction of vitamin A palmitate preparations. In the case of the syrup preparation, the nonisothermal method provided the shelf-life estimates, $t_{90(25)}$ and $t_{90(40)}$, closer to the value measured experimentally at 25 and 40°C than the isothermal method carried out at 40, 50, 60, and 70°C. The nonisothermal method may have an advantage in providing better estimates of shelf lives than the isothermal method when drug degradation does not follow the Arrhenius equation near ambient conditions. Even such a small change in E_a with temperature as shown for the vitamin A preparations, which is usually overlooked, might bring about a large bias of estimates in the isothermal method. In this case, the nonisothermal method can give shelf-life estimates with smaller bias because data collected near room temperature are partially involved in the method. In other words, the bias of the estimates brought about by neglecting the nonlinearity of the Arrhenius plots was much smaller in the nonisothermal method than in the isothermal method. This is noteworthy because the E_a of drug degradation often varies within the temperature range studied for stability tests.

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